

ORIGINAL ARTICLE

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Applications of self-organizing map for patterning macrofungal diversity of xerothermic swards

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Abstract Macrofungal communities were investigated in four associations of xerothermic swards: *Festucetum pal-lentis*, *Origano-Brachypodietum*, *Adonido-Brachypodietum pinnati* and *Diantho-Armerietum elongatae* in a Jurassic area of the Częstochowa Upland (southern Poland). A total of 47 species were recorded. The self-organising map (SOM)—an unsupervised algorithm for artificial neural networks—was used to recognise patterns in the macrofungal communities of diverse xerothermic swards. Only two associations were mycologically similar: *Origano-Brachypodietum* and *Adonido-Brachypodietum pinnati*. Species with high and significant IndVal (the species indicator value) for each investigated phytocoe-noses are presented. The presence of macrofungal species and the participation of indicator species were connected with habitat factors of plant associations, as documented by the IndVal application. In the least fertile phytocoe-noses, macrofungal communities were poor with few indicator species. The more fertile phytocoenoses had richer and more varied communities of macrofungi with higher numbers of indicator species. The ordering meth-ods applied in this study were very effective for analyzing the macrofungal communities existing in plant associa-tions.

Keywords Fungi · Xerothermic vegetation · Habitat factor · Self-organizing map · IndVal

Introduction

Macrofungal communities inhabiting plant associations have been the subject of much research but xerothermic swards have been examined in three localities only (Wojewoda 1974, 1975, 1977; Stasińska 2005; Adamczyk

2009). However, gaining knowledge on these associations is indispensable because they are seriously threatened in Poland and other European countries (Olaczek 1998). The list of macromycete species found in the swards of the *Festuco-Brometea* class, compiled from literature from the latest 50 years, includes only about 40 taxa (Stasińska 2008). Xerothermic swards are mentioned among habi-tats of especial importance in the Habitats Directive (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and wild fauna and flora). It is a well known fact that xerothermic swards are seminatural and, just for that reason, do not form stable communities. They require the constant human activity afforded by grazing by cattle and mowing (Olaczek 1998). Any attempt to widen our knowledge of xerothermic swards could be useful for their protection. Distinguishing indicator species of macromycetes for different xero-thermic swards could help us to estimate changes in these plant communities, because fungi react to habitat changes more quickly than plants (Arnolds 1981).

Macromycete research on plant communities of Europe relies mainly on the phytosociological Braun-Blanquet method (e.g., Ławrynowicz 1973; Wojewoda 1975; Arnolds 1994). Suitable statistical methods, such as DCA, TWINSpan, and ACC have been applied in only a few studies (e.g., Adamczyk et al. 2004; Fraiture 2004). The Braun-Blanquet method proposed for plants permits the abundance and constancy of species at a designated site to be estimated. The method is under-stood rather as a plant abundance measure, as fungus fruiting body numbers do not give an indication of fungus mycelia volume (Fraiture 2008). Beside, the carpophores of one species noted on a permanent plot could be produced by different mycelia. Therefore, the frequency of macrofungal species is more significant than the number of specimens, because it indicates the occurrence of species in that community. Hence, my search for a method that will allow an objective analysis of relationships between macrofungi and plant com-munities of separating species, which is particularly significant for different phytocoenoses.

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The self-organizing map (SOM) algorithm and the IndVal index have not yet been applied to macromycetes communities. In this study, the SOM was used for the following reasons: (1) macromycetes community studies already published in Poland did not reveal any association between such communities and xerothermic swards; (2) the availability of very rich data means that symbols and their codes are usually illegible because they overlap (Kruk et al. 2007); and (3) we also cannot determine from gradient analysis where the border between clusters of samples or species is located in multivariate space (Cho 1997).

Xerothermic swards are found in Southern and Southeastern Europe in areas with a warm, dry climate, and they also occur extrazonally in places with a particular combination of orographic, soil and local climatic factors (Matuszkiewicz 2001). Xerothermic plant species most likely entered Poland during the Elsterian glaciation from three directions: from the Hungarian lowland through the Moravian Gate; from the south-west through Podolye; and from the southeast, i.e., from Turingia (Krzakowa and Michalak 2007). In Poland, these rare phytocoenoses are currently situated in southern and north-western parts of the country. They develop in locations characterized by high temperatures and high calcium carbonate content in the soil. Plant communities developing in such specific biotopic conditions are distinguished by characteristic species compositions with many rare taxa (Szafer 1977; Matuszkiewicz 2001).

The principal aims of this study were (1) to examine the mycological similarity of diverse xerothermic swards, and (2) to determine the indicator species (IndVal) of macrofungi for each xerothermic sward.

Materials and methods

Study site

This study was conducted from 1997 to 2000 and from 2002 to 2003 in a region of the Częstochowa Upland. The Częstochowa Upland is located in southern Poland and occupies an area of 1,299 km² (Kondracki 1998; Dylikowa 1973). The landscape is characterized by limestone rocky crags that often have castle ruins positioned on top of them, as well as numerous caves and erosion valleys. The Mesozoic base of the Częstochowa Upland consists of Upper Jurassic limestone covered by Pleistocene deposits (Dylikowa 1973). This area has an extraordinarily rich and varied flora, including mountain and thermophilic plants, relicts and even endemics; e.g., *Galium cracoviense*, *Saxifraga aizoon*, *Botrychium lunaria* and *Anemone sylvestris* are found here (Hereźniak 2002, 2004).

The investigated xerothermic swards are situated in the northern part of the Częstochowa Upland on six calcareous hills (50°45'07"N 19°16'04"E). Four different associations of semi-natural dry grasslands were

examined: *Festucetum pallentis* (FP), *Origano-Brachypodium* (OBr), *Adonido-Brachypodium pinnati* (AB), and *Diantho-Armeriatum elongatae* (DA).

The FP association develops on steep, calcareous rocks, where soil occurs only in the interstices between rocks. The soil is rather poor in humus but rich in calcium carbonate (about 7%). The species *Festuca pallens*, *Thymus pulegioides*, *Sedum acre*, *Galium cracoviense* and *Tortula ruralis* dominate the floristic composition. The coverage of the plant layer is low (about 30%).

The OBr and AB associations develop in soil with a well developed humus horizon and calcium carbonate content equal to approximately 8%. The vegetation coverage of this plant association is up to 100%, and it forms high, colourful swards of several layers. Different species of grasses and sedges grow here, e.g., *Brachypodium pinnatum*, *Festuca ovina*, *Agrostis canina* and *Carex caryophylla*. These phytocoenoses have a distinctive composition of shrubs and trees, e.g., *Juniperus communis*, *Rhamnus cathartica*, *Corylus avellana* and *Sorbus aucuparia*.

The DA association occurs on sandy soils that are poor in calcium carbonate. The vegetation coverage of this community is high (70–100%). The floristic composition is dominated by *Cerastium arvense*, *Armeria elongata*, *Dianthus deltoides*, *Corynephorus canescens*, *Scleranthus perennis*, *Festuca rubra* and *Anthoxanthum odoratum* (Babczyńska 1978).

Collection of samples

In four associations of xerothermic swards, ascomycete and basidiomycete macrofungi were observed in a total of 30 permanent plots (the area of each plot was 4–50 m²). The size of plots and their locations were taken from the phytosociological classification by Babczyńska (1978) and Babczyńska-Sendek (1984). The presence of fungus in each site was examined every 3 weeks on 28 occasions from April to November 1997–2000 and 2002–2003. Herbarium specimens were deposited with the University Herbarium of Łódź. The species nomenclature of *Ascomycota* followed Dennis (1978) and Chmiel (2006), and the species nomenclature of *Basidiomycota* was taken from Wojewoda (2003).

Statistical data analysis

An unsupervised algorithm of an artificial neural network, i.e., a SOM, was used to recognize patterns in the macrofungi in the study area (Kohonen 1982). The advantage of this method is that it can be used to effectively analyze complex data sets despite non-linear relationships and non-normal distributions, and it results in a two-dimensional map that is easy to interpret (Kohonen 1982). The SOM has proved to be an effective and powerful tool for exploring patterns in species distributions, and the structure of communities (Chon et al.

1996; Giraudel and Lek 2001; Penczak et al. 2005, 2009; Kruk 2006; Lasne et al. 2007; Kalteh et al. 2008; Bedoya et al. 2009). The SOM was simulated and cluster analysis was performed in Matlab (ver. 6.1.0.450). A Kohonen neural network was built of two layers of neurons (processing units): an input layer and an output layer. The number of input neurons was equal to the number of variables in the data set, i.e., the number of fungal species (47). Each input neuron was connected to all of the output neurons. During the training process, input neurons transmit signals to the output layer of neurons. The data (47 taxa \times 30 sites) were displayed on the input layer of neurons (i.e., processing units) 180 times in the rough training phase, and 660 times in the finetuning phase. Output layers of different sizes were examined step by step from 4×4 (16 neurons) to 8×8 (64 neurons), and an output layer of 4×4 neurons was ultimately chosen. To finally select a map size, I use the heuristic rule of Versanto et al. (2000): the map unit is $5\sqrt{n}$, where n is the number of training samples. The actual number of neurons should be close to the number determined by Versanto's rule, but I have accepted a slightly smaller map because a 5×5 map had many empty units, which is not helpful (C  r  ghino and Park 2009).

Log transformed and normalized (0–1) numbers of individuals were presented to the neurons in the input layer. A batch training algorithm was used, which is significantly faster and does not require specification of any learning rate factor, and the results of the batch algorithm are not dependent on the learning order of inputs (Park et al. 2006). Generally, the SOM is a robust and useful method for ecological studies (Kalteh et al. 2008), and different training functions do not have any noticeable effect on its operation (Noori et al. 2010).

The map obtained after the training process of the SOM contained all the macrofungi sites assigned to neurons so that similar sites were located in one neuron or in adjoining neurons, and significantly dissimilar sites were in distant neurons (Chon et al. 1996; Park et al. 2003; Bedoya et al. 2009). To subdivide the output neurons into different clusters (groups) according to their similarity (Bedoya et al. 2009), I used a hierarchical cluster analysis with the Ward linkage method and Euclidean distance measurements.

To show which cluster of neurons (and their environmental conditions) was most preferred by each species, the indicator value (IndVal) defined by Dufrene and Legendre (1997) was used. This value was obtained for each species from the original untransformed data matrix (number of individuals) and was attributed to the clusters distinguished by SOM. The species that presented significant values of IndVal in any cluster were then selected using Monte Carlo statistics. The above calculations were made using PC-ORD statistical software (MacCune and Mefford 1992). To avoid drawing invalid conclusions, only species having IndVal > 25 were taken into consideration. IndVals complement the information presented on graphs of species importance

(component planes) produced by the SOM (Lasne et al. 2007; Penczak et al. 2009).

Results

A total of 47 species of macrofungi were found in the investigated xerothermic swards. The sites richest in macrofungi were located in the *Origano-Brachypodium* (39 species) and *Adonido-Brachypodium pinnati* associations (37 species). The poorest sites were situated in the *Festuco pallentis* association (13 species) (Table 1).

The trained SOM indicated three main clusters of sites assigned to the output neurons: (X) A1, A2, A3, A4, B2, B3 and B4; (Y1) B1, C1, C2, D1; and (Y2) C2, C3, C4, D2, D3 and D4 (Fig. 1). The clusters X and Y1 were homogeneous within themselves but differed from each other, essentially in the character of sites assigned to them. Cluster X contains all 15 sites from the FP association (neurons A2, A3, A4); cluster Y1 contains all 5 sites located in DA. These sites are situated in only one neuron (C1) and separated by neighboring, wide hexagons B1 and C2 from cluster X and C2 and D1 from cluster Y1. Cluster Y2 is less homogeneous because it contains ten sites of two diverse xerothermic swards: OBr and AB, located in three neurons: D2, D3 and D4. The sites of these two associations are mixed in two neurons, D3 and D4. Only one site, AB81, is situated in neuron D2. The wide neurons separate these sites from cluster X (C3, C4).

The IndVal showed the importance of all species of macrofungi on the SOM (Fig. 2; Table 2). Species with a high and significant IndVal for cluster X included *Arrhenia spathulata*, *Hemimycea mairei* ($P < 0.001$) and *Geastrum minimum* ($P < 0.01$). The indicator species for cluster Y1 included the following: *Aleuria aurantia*, *Coprinus atramentarius*, *C. comatus*, *Scleroderma bovista*, *S. verrucosum* and *Vascellum pratense* ($P < 0.001$); *Bovista nigrescens* and *Melanoleuca brevipes* ($P < 0.01$); and *Bovista graveolens*, *Lycoperdon molle* and *Marasmius oreades* ($P < 0.05$).

Cluster Y2 was the richest in species with significant IndVal values and included the following: *Agrocybe pediades*, *A. praecox*, *Calocybe gambosa*, *Calvatia utriformis*, *Camarophyllus pratensis*, *C. virgineus*, *Hygrocybe persistans*, *Lycoperdon ericaeum* and *Tubaria furfuracea* ($P < 0.001$); *Crinipellis scabellata*, *Lycoperdon decipiens*, *Marasmius scorodoni* and *Rickenella fibula* ($P < 0.01$); and *Calvatia excipuliformis*, *Hygrocybe psittacina*, *Panaeolus fimicola*, *P. sphinctrinus*, *Rickenella setipes*, *Telephora caryophyllaea*, *Volvariella bombycina* and *V. pusilla* ($P < 0.05$).

Discussion

Macrofungal communities had different species structures in diverse xerothermic swards. The macrofungal communities of FP and DA associations were the most

Table 1 Frequency of presence of macrofungal species on investigated sites with four different associations of semi-natural dry grasslands

Macrofungal species		Site																													
		FP 63	FP 66	FP 70	FP 68	FP 74	FP 73	FP 62	FP 64	FP 61	FP 71	FP 69	FP 72	FP 75	FP 65	FP 67	AB 82	AB 85	AB 83	AB 84	AB 81	OBr 78	OBr 80	OBr 77	OBr 76	OBr 79	DA 89	DA 87	DA 86	DA 88	DA 90

FP *Festucetum pallentis*, AB *Adonido-Brachypodietum*, OBr *Origano-Brachypodietum pinnati*, AD *Diantho-Armerietum elongatae*

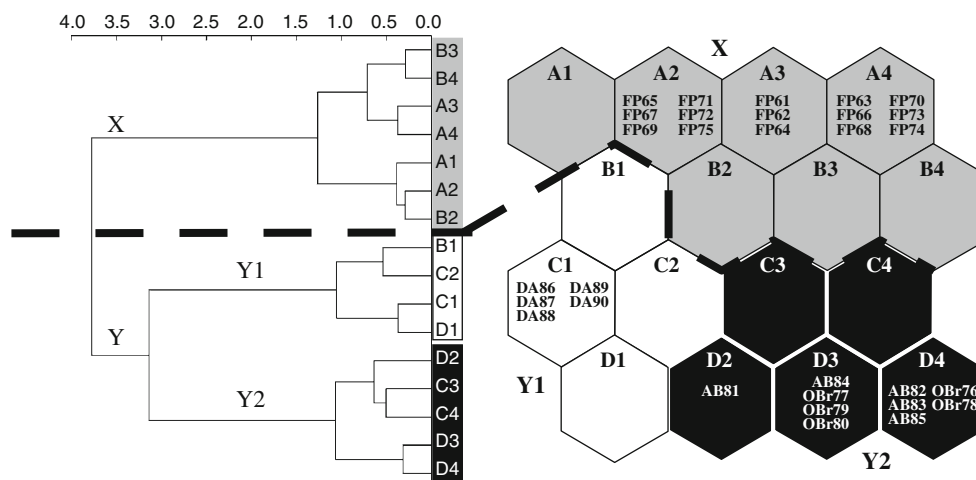


Fig. 1 The 30 sites from xerothermic swards assigned to self-organizing map (SOM) neurons. Sites are marked with codes, as explained in Table 1. Distance for clusters on the Ward diagram are marked on the y-axis

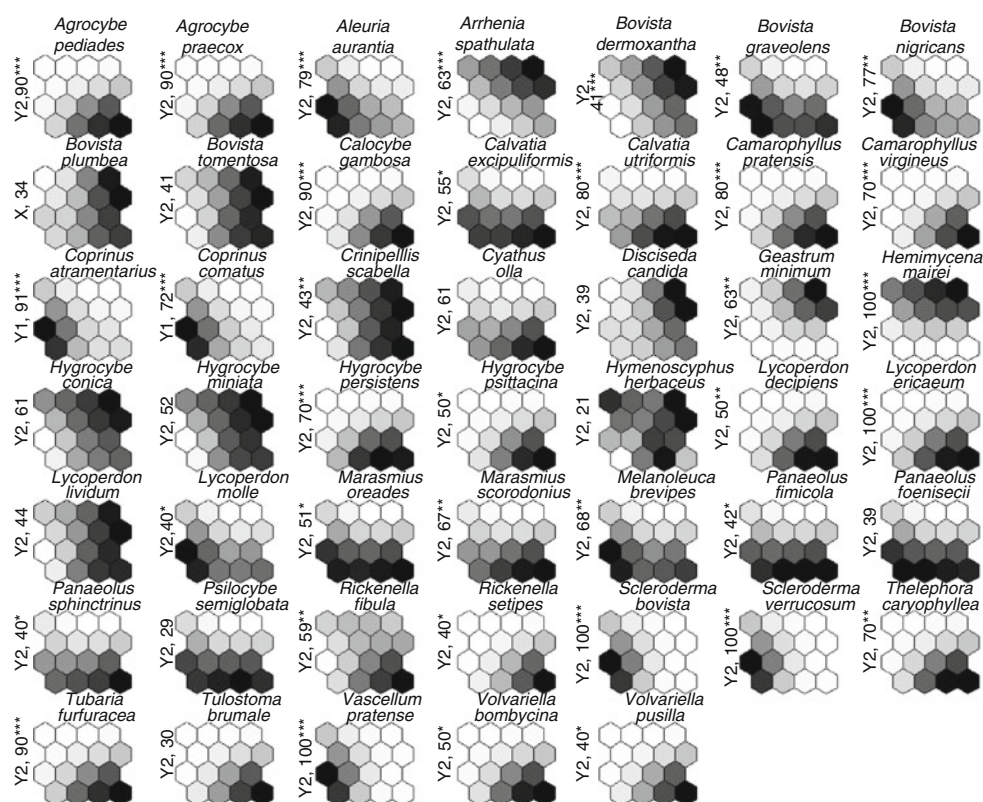


Fig. 2 The importance of the macrofungal species in the SOM according to the most activated regions of the SOM and the IndVal index. The left margin of each figure gives an indicator value (IndVal), and its significance level for a given cluster is marked: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

different. The mycobiotes of OBr and AB associations were very similar (Fig. 1).

The FP sites were characterized by very poor species composition of macrofungal communities (Table 1). They consist of fungi growing on mosses (*Arrhenia spathulata*) and grass (*Hemimycena mairei*), which produce small fruiting bodies, and some xerophilous species, e.g., *Disciseda candida*, *Bovista plumbea* and *B. tomentosa*.

Sites of dense xerothermic swards (OBr and AB) were richest in species of macromycetes. The majority of macrofungus species occur on these xerothermic swards (e.g., *Calocybe gambosa*, *Camarophyllus pratensis*, *C. virgineus*, *Hygrocybe persistens*, *Mariasmius oreades*, *Panaeolus foemiseccii*) were noted by various authors (e.g., Arnolds 1981; Gumińska 1997; Adamczyk et al. 2004) from the plant communities of the alliances

Table 2 Species distinguished by the IndVal index as significant in self-organizing map (SOM) clusters for xerothermic swards of the Czystochowa Upland. The IndVal value is indicated after the species name

Clusters		
X	Y1	Y2
<i>Arrhenia spathulata</i> 63***	<i>Aleuria aurantia</i> 79***	<i>Agrocybe pediades</i> 90***
<i>Geastrum minimum</i> 60**	<i>Bovista graveolens</i> 48*	<i>Agrocybe praecox</i> 90***
<i>Hemimycena mairei</i> 100***	<i>Bovista nigricans</i> 77**	<i>Calocybe gambosa</i> 90***
	<i>Coprinus atramentarius</i> 91***	<i>Calvatia excipuliformis</i> 55*
	<i>Coprinus comatus</i> 72***	<i>Calvatia utriformis</i> 80***
	<i>Lycoperdon molle</i> 40*	<i>Camarophyllus pratensis</i> 80***
	<i>Marasmius oreades</i> 51*	<i>Camarophyllus virgineus</i> 70***
	<i>Melanoleuca brevipes</i> 68**	<i>Crinipellis scabella</i> 43**
	<i>Scleroderma bovista</i> 100***	<i>Hygrocybe persistens</i> 70***
	<i>Scleroderma verrucosum</i> 100***	<i>Hygrocybe psittacina</i> 50*
	<i>Vascellum pratense</i> 100***	<i>Lycoperdon decipiens</i> 50**
		<i>Lycoperdon ericaeum</i> 100***
		<i>Marasmius scorodoni</i> 67**
		<i>Panaeolus fimicola</i> 42*
		<i>Panaeolus sphinctrinus</i> 40*
		<i>Rickenella fibula</i> 59**
		<i>Rickenella setipes</i> 40*
		<i>Thelephora caryophyllea</i> 70**
		<i>Tubaria furfuracea</i> 90***
		<i>Volvariella bombycina</i> 50*
		<i>Volvariella pusilla</i> 40*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Arrhenatherion elatoris and *Cynosurion*. There are also a few xerophilous, saprobic species (e.g., *Bovista graveolens*, *B. tomentosa*, *Lycoperdon molle*) and one mycorrhizal species, *Thelephora caryophyllea*. Macromycetes of OBr and AB associations contain several species observed in steppe habitats, such as *Agrocybe pediades*, *Bovista plumbea*, *Calvatia utriformis* and *Coprinus comatus* (Kenan and Yuzuf 2002). Macrofungi of the DA association are mainly xerophilous species from the genera *Scleroderma*, *Bovista*, *Lycoperdon* and *Vascellum*, while macrofungi from the genus *Coprinus* were observed in many different (natural or anthropogenic) habitats.

The richness of macrofungal communities in each xerothermic sward association and the presence of indicator species (with significant IndVal) of macrofungi are connected with different habitat factors in the investigated phytocoenoses. The *Festucetum pallentis* association is characterized by a thin humus layer and low humidity (Babczyńska 1978). These conditions are preferred by a very small group of macrofungal species. Most of these species were only found in this association (Adamczyk 2009; Wojewoda 1974, 1975). However, *Arrhenia spathulata*—a species associated with mosses—was found in a variety of locations. *Arrhenia spathulata* appears to be a species that grows often on poor habitats; e.g., it was noted on mosses in the sandy dunes of Sicily (Lantieri et al. 2008). Two phytosociologically similar plant associations, *Origano-Brachypodietum* and *Adonido-Brachypodietum pinnati*, are located in areas with higher humidity and the richest soils. They are preferred by a large group of macrofungi comprising less specialized species that are found in other plant associations. *Diantho-Armerietum elongatae* occur on sandy,

poor soils (Babczyńska 1978). This habitat is preferred by typically xerophilous macrofungi (Table 2). Interestingly, a majority of indicator species in xerothermic swards are saprotrophic macrofungi. Only *Scleroderma bovista*, *S. verrucosum* and *Thelephora caryophyllea* are mycorrhizal species. *Geastrum minimum* and macrofungi from the genus *Hygrocybe* may create symbiotic relations with trees (Wojewoda 2003), but this has not been confirmed. One species, *Rickenella fibula*, parasitizes mosses (Elborn 2008).

The present research on macrofungal communities confirmed the phytosociological differences between the *Festucetum pallentis* association and other associations from the class *Festuco-Brometea*. The results also showed that communities of macrofungi with close associations in terms of phytosociology are very similar, e.g., *Origano-Brachypodietum* and *Adonido-Brachypodietum pinnati*.

Thus far, the studies on xerothermic swards (e.g., Stasińska 2005; Wojewoda 1975) have analyzed macrofungi from the mycoenological point of view. These studies did not show the differences between macrofungal communities in different associations of xerothermic swards. In my work, clustering of macrofungi samples in xerothermic swards showed clearly that the structure of macrofungal communities depends on the type of plant association. Macrofungal communities can be good indicators of the habitat diversity of these plant associations; they may also be used to identify modified, degraded and disturbed sites and thus to evaluate phytocoenoses. Using the SOM, both ecological assessments can be conducted easily (Kruk 2006) at various spatial scales. Additionally, the grouping of macrofungi

samples by SOM has certain advantages: (1) it does not require a priori knowledge about species requirements; and (2) it takes into consideration all species.

Studies by Magura et al. (2001, 2002) and Whitehouse and Bayley (2005) concluded that immobile organisms are characterized by high and stable IndVal values. This pattern was confirmed by the present research. The majority of recognized macrofungal species (73%) had high IndVal numbers. This suggests a very important role of macrofungi in semi-natural, unstable phytocoenoses, whose existence depends on intensive human activity (exploitation as pastures, and mowing of grasses). The IndVal is a particularly effective tool for ecological bioindication (MacGeoch et al. 2002; Paavola et al. 2003; Penczak 2009). The results of the present mycological investigations of xerothermic swards demonstrate the value of macrofungi as bioindicators that can be useful in the practical conservation of rare and endangered plant communities.

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